Protein databases

Henrik Nielsen

Protein databases, historical background

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Swiss-Prot, http://www.expasy.org/sprot/

Established in 1986 in Swizerland

ExPASy (Expert Protein Analysis System)

Swiss Institute of Bioinformatics (SIB) and European Bioinformatics Institute (EBI)

PIR, http://pir.georgetown.edu/

Established in 1984

National Biomedical Research Foundation, Georgetown University, USA

In 2002 merged into:

UniProt, http://www.uniprot.org/

A collaboration between SIB, EBI and Georgetown University.

UniProt

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UniProt Knowledgebase (UniProtKB)

UniProt Reference Clusters (UniRef)

UniProt Archive (UniParc)

UniProt Knowledgebase Release 2012_05 (May 16, 2012) consists of:

UniProtKB/Swiss-Prot: Annotated manually (*curated*)

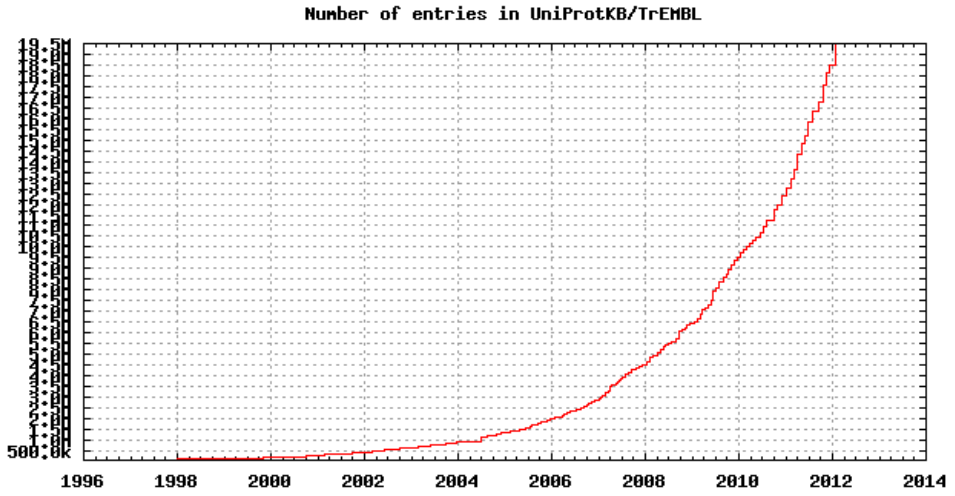
536,029 entries, 190,235,160 amino acids

UniProtKB/TrEMBL: Computer annotated (automatically translated from nucleotide databases)

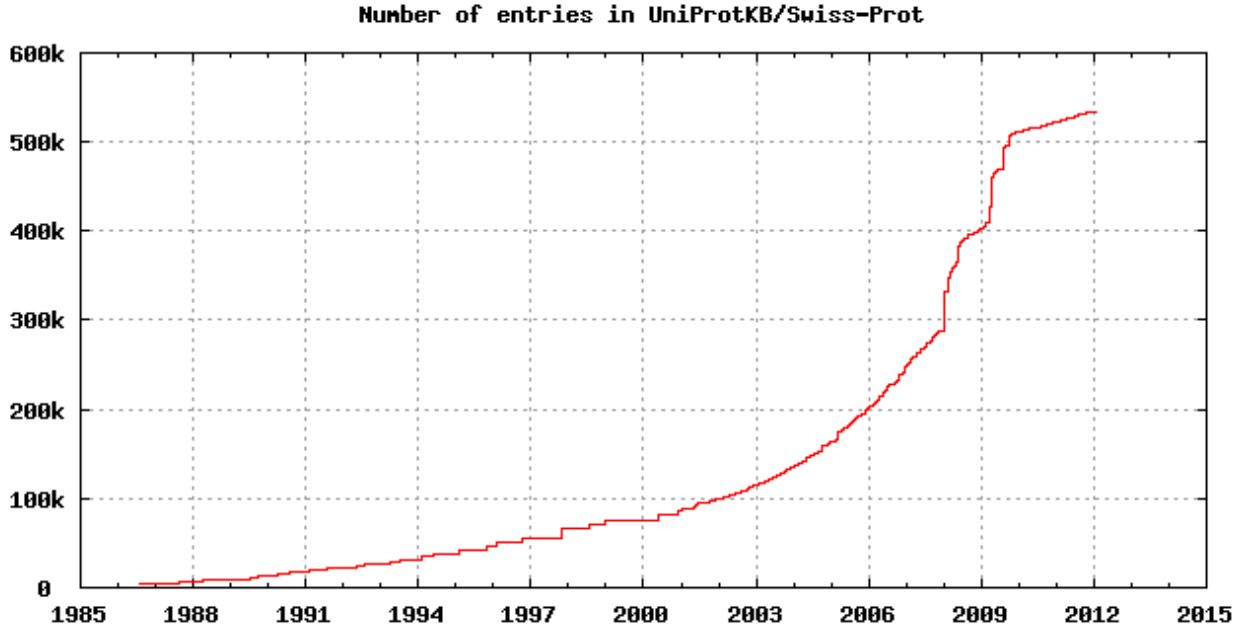
22,128,511 entries, 7,226,807,757 amino acids

Growth of UniProt

TrEMBL



Swiss-Prot



Content of UniProt Knowledgebase

- Amino acid sequences
- Functional and structural annotations
  - Function / activity
  - Secondary structure
  - Subcellular location
  - Mutations, phenotypes
  - Post-translational modifications
- Origin
  - organism: Species, subspecies; classification
  - tissue
- References
- Cross references

Amino acid sequences

From where do you get amino acid sequences?

- Translation of nucleotide sequences (GenBank/EMBL/DDBJ)
- Direct amino acid sequencing: *Edman degradation*
- Mass spectrometry
- 3D-structures

UniProt entry, formatted view

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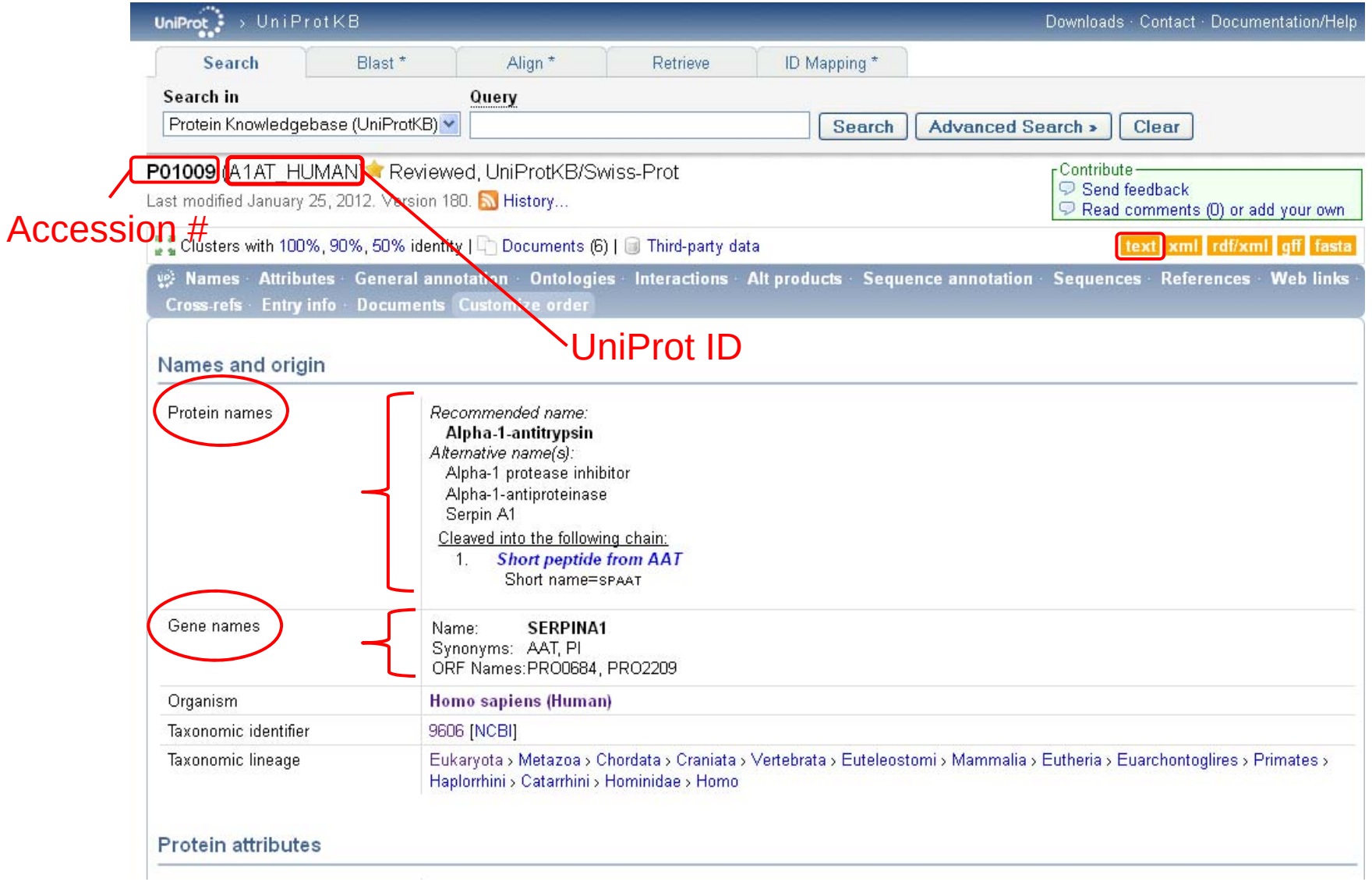

UniProt entry, text view (flat file)


```
ID   A1AT_HUMAN              Reviewed;         418 AA.
AC   P01009; A6PX14; B2RDQ8; Q0PVP5; Q13672; Q53XB8; Q5U0M1; Q7M4R2;
AC   Q86U18; Q86U19; Q96BF9; Q96ES1; Q9P1P0; Q9UCE6; Q9UCM3;
DT   21-JUL-1986, integrated into UniProtKB/Swiss-Prot.
DT   01-OCT-1996, sequence version 3.
DT   25-JAN-2012, entry version 180.
DE   RecName: Full=Alpha-1-antitrypsin;
DE   AltName: Full=Alpha-1 protease inhibitor;
DE   AltName: Full=Alpha-1-antiproteinase;
DE   AltName: Full=Serpin A1;
DE   Contains:
DE     RecName: Full=Short peptide from AAT;
DE              Short=SPAAT;
DE   Flags: Precursor;
GN   Name=SERPINA1; Synonyms=AAT, PI; ORFNames=PRO0684, PRO2209;
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
OC   Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   NUCLEOTIDE SEQUENCE [MRNA] (ISOFORM 1).
RX   MEDLINE=84107980; PubMed=6319097;
RA   Bollen A., Herzog A., Cravador A., Herion P., Chuchana P.,
RA   van der Straten A., Loriau R., Jacobs P., van Elsen A.;
RT   "Cloning and expression in Escherichia coli of full-length
RT   complementary DNA coding for human alpha 1-antitrypsin.";
RL   DNA 2:255-264(1983).
...
```

General annotation (Comments)

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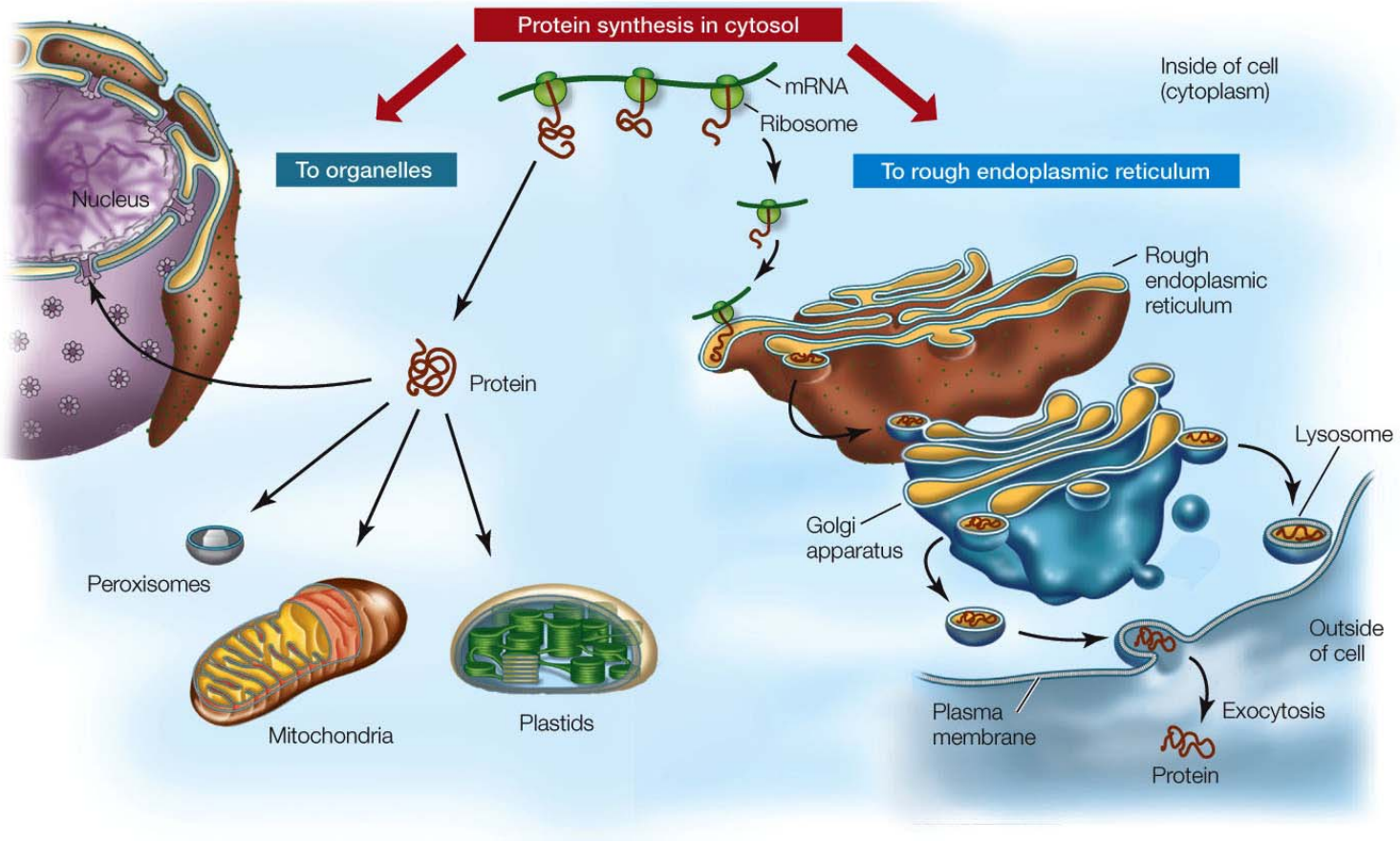
General annotation (Comments)

Function	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin. Irreversibly inhibits trypsin, chymotrypsin and plasminogen activator. The aberrant form inhibits insulin-induced NO synthesis in platelets, decreases coagulation time and has proteolytic activity against insulin and plasmin. Ref.17 Ref.18 Ref.24 Short peptide from AAT (SPAAT) is a reversible chymotrypsin inhibitor. It also inhibits elastase, but not trypsin. Its major physiological function is the protection of the lower respiratory tract against proteolytic destruction by human leukocyte elastase (HLE). Ref.17 Ref.18 Ref.24
Subcellular location	Secreted Ref.24. Short peptide from AAT: Secreted › extracellular space › extracellular matrix Ref.24.
Tissue specificity	Plasma.
Domain	The reactive center loop (RCL) extends out from the body of the protein and directs binding to the target protease. The protease cleaves the serpin at the reactive site within the RCL, establishing a covalent linkage between the carboxyl group of the serpin reactive site and the serine hydroxyl of the protease. The resulting inactive serpin-protease complex is highly stable.
Post-translational modification	Several isomers are observed, resulting from the combination of different N-linked glycan structures and mature N-terminus. N-linked glycan at Asn-107 is alternatively di-antennary, tri-antennary or tetra-antennary, whereas glycan at Asn-70 is di-antennary with trace amounts of tri-antennary, and glycan at Asn-271 is exclusively di-antennary. The structure of the antennae is Neu5Ac(alpha1-6)Gal(beta1-4)GlcNAc attached to the core structure Man(alpha1-6)[Man(alpha1-3)]Man(beta1-4)GlcNAc(beta1-4)GlcNAc. Some antennae are fucosylated, which forms a Lewis-X determinant. Proteolytic processing may yield the truncated form that ranges from Asp-30 to Lys-418.
Polymorphism	The sequence shown is that of the M1V allele which is the most common form of PI (44 to 49%). Other frequent alleles are: M1A 20 to 23%; M2 10 to 11%; M3 14 to 19%.
Involvement in disease	Defects in SERPINA1 are the cause of alpha-1-antitrypsin deficiency (A1ATD) [MIM:613490]. A disorder whose most common manifestation is emphysema, which becomes evident by the third to fourth decade. A less common manifestation of the deficiency is liver disease, which occurs in children and adults, and may result in cirrhosis and liver failure. Environmental factors, particularly cigarette smoking, greatly increase the risk of emphysema at an earlier age. Ref.58 Ref.60 Ref.62
Miscellaneous	The aberrant form is found in the plasma of chronic smokers, and persists after smoking is ceased. It can still be found ten years after smoking has ceased.
Sequence similarities	Belongs to the serpin family.
Sequence caution	The sequence CAD62334.1 differs from that shown. Reason: Erroneous initiation. Translation N-terminally shortened. The sequence CAD62585.1 differs from that shown. Reason: Erroneous initiation. Translation N-terminally shortened.

Protein sorting in eukaryotes



Different proteins belong to different compartments of the cell – and some belong *outside* the cell

General annotation (Comments)

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General annotation (Comments)

Function	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin. Irreversibly inhibits trypsin, chymotrypsin and plasminogen activator. The aberrant form inhibits insulin-induced NO synthesis in platelets, decreases coagulation time and has proteolytic activity against insulin and plasmin. Ref.17 Ref.18 Ref.24 Short peptide from AAT (SPAAT) is a reversible chymotrypsin inhibitor. It also inhibits elastase, but not trypsin. Its major physiological function is the protection of the lower respiratory tract against proteolytic destruction by human leukocyte elastase (HLE). Ref.17 Ref.18 Ref.24
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Miscellaneous	The aberrant form is found in the plasma of chronic smokers, and persists after smoking is ceased. It can still be found ten years after smoking has ceased.
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Sequence caution	The sequence CAD62334.1 differs from that shown. Reason: Erroneous initiation. Translation N-terminally shortened. The sequence CAD62585.1 differs from that shown. Reason: Erroneous initiation. Translation N-terminally shortened.

Post-translational modifications

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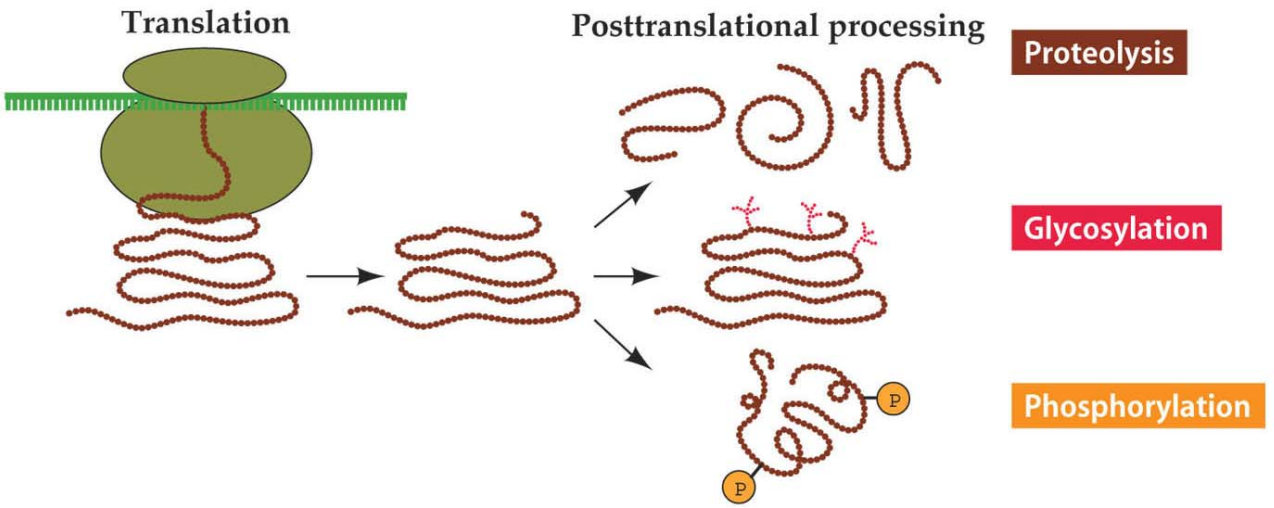


Many proteins need to be *modified* after their synthesis to become active

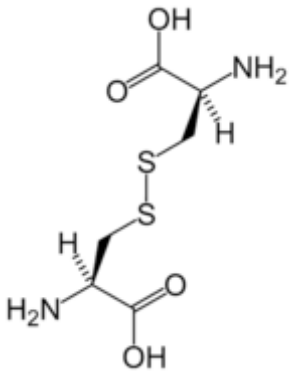
Proteolysis: cleavage of *signal peptides*, *propeptides* or *initiator methionine*

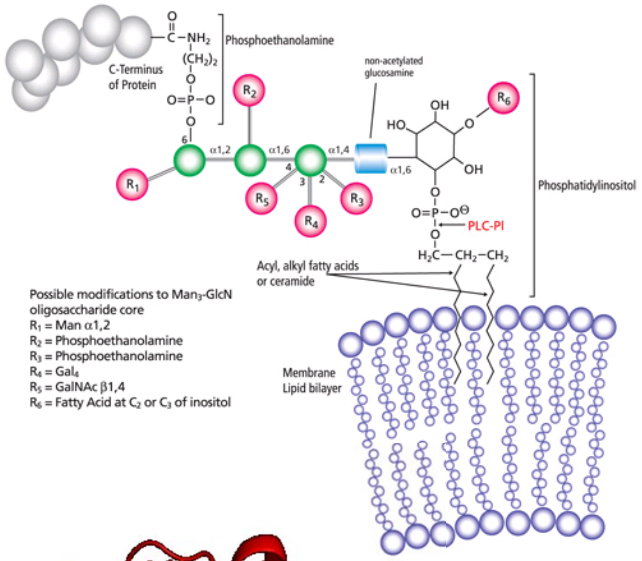
Glycosylation: Especially relevant on the cell *surface.* Also plays a role in sorting of proteins to *lysosomes*

Phosphorylation: Often *reversible*. Regulates the *activity* of many enzymes

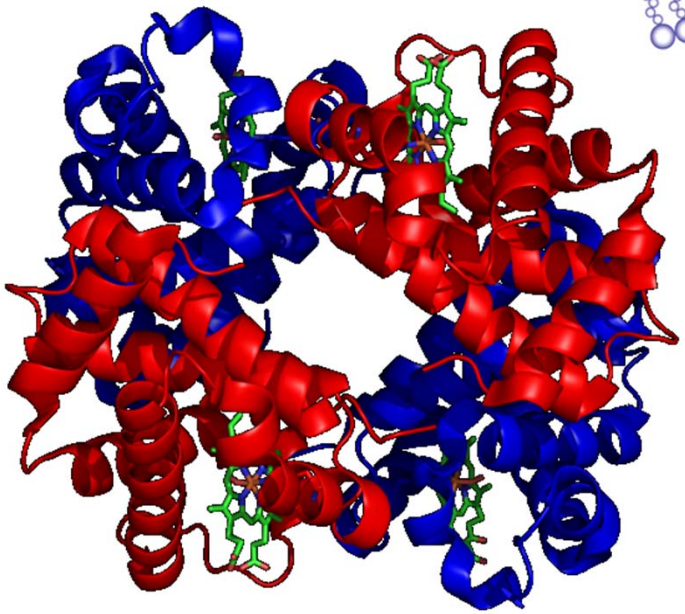
More post-translational modifications

- Lipid anchors
  - (e.g. GPI anchors)
- Disulfide bonds





- Prosthetic groups
  - (*e.g.* metal ions)



General annotation (Ontologies)

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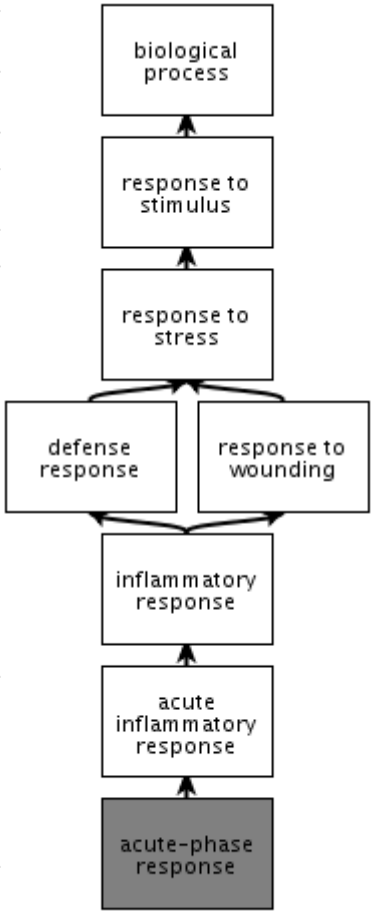
Ontologies

Keywords

Biological process	Acute phase Blood coagulation
Cellular component	Extracellular matrix Secreted
Coding sequence diversity	Alternative splicing Polymorphism
Domain	Signal
Molecular function	Protease inhibitor Serine protease inhibitor
PTM	Glycoprotein
Technical term	3D-structure Complete proteome Direct protein sequencing Reference proteome

Gene Ontology (GO)

Biological process	acute-phase response Inferred from electronic annotation. Source: UniProtKB-KW platelet activation Traceable author statement. Source: Reactome platelet degranulation Traceable author statement. Source: Reactome regulation of proteolysis Inferred from Biological aspect of Ancestor. Source: RefGenome
Cellular component	extracellular space Inferred from mutant phenotype. Source: UniProtKB platelet alpha granule lumen Traceable author statement. Source: Reactome proteinaceous extracellular matrix Inferred from electronic annotation. Source: UniProtKB-SubCell
Molecular function	protease binding Inferred from physical interaction. Source: UniProtKB serine-type endopeptidase inhibitor activity Non-traceable author statement. Source: UniProtKB



QuickGO - http://www.ebi.ac.uk/QuickGO

Sequence annotation (Feature Table)

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Sequence annotation (Features)

	Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Molecule processing						
☐	Signal peptide	1 – 24	24	Ref.15 Ref.16 Ref.17 Ref.18		
☐	Chain	25 – 418	394	Alpha-1-antitrypsin Ref.2		PRO_0000032377
☐	Peptide	375 – 418	44	Short peptide from AAT		PRO_0000364030
Regions						
☐	Region	368 – 392	25	RCL		
Sites						
☐	Site	382 – 383	2	Reactive bond		
Amino acid modifications						
☐	Modified residue	256	1	S-cysteinyl cysteine		
☐	Glycosylation	70	1	N-linked (GlcNAc...) (complex) Ref.19 Ref.26 Ref.27 Ref.28 Ref.29 Ref.30 Ref.31 Ref.32		
☐	Glycosylation	107	1	N-linked (GlcNAc...) (complex) Ref.19 Ref.29 Ref.31 Ref.32		
☐	Glycosylation	271	1	N-linked (GlcNAc...) (complex) Ref.19 Ref.26 Ref.27 Ref.28 Ref.29 Ref.31 Ref.32		
Natural variations						
☐	Alternative sequence	307 – 418	112	Missing in isoform 3.		VSP_028890
☐	Alternative sequence	356 – 418	63	AVHKA...NPTQK → VRSP in isoform 2.		VSP_028889
☐	Natural variant	4	1	S → L in Z-Wrexham. Ref.62		VAR_006978
☐	Natural variant	26	1	D → A in V-Munich. Ref.59		VAR_006979
☐	Natural variant	37	1	T → A. Corresponds to variant rs11558262 [dbSNP | Ensembl].		VAR_051938
☐	Natural variant	58	1	A → T in M5-Karlsruhe.		VAR_006980
☐	Natural variant	63	1	R → C in I. Ref.63 Corresponds to variant rs28931570 [dbSNP | Ensembl].		VAR_006981

Secondary structure (Feature Table)

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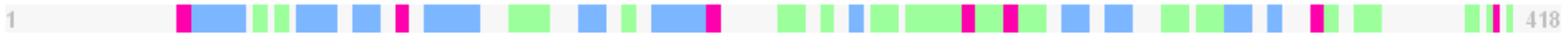
Secondary structure



Helix Strand Turn

Details...

☐	Turn	48 – 50	3
☐	Helix	51 – 68	18
☐	Beta strand	70 – 72	3
☐	Beta strand	74 – 76	3
☐	Helix	78 – 89	12
☐	Helix	94 – 103	10
☐	Turn	108 – 110	3
☐	Helix	113 – 127	15
☐	Beta strand	135 – 145	11
☐	Helix	152 – 160	9
☐	Beta strand	165 – 169	5
☐	Helix	174 – 188	15
☐	Turn	189 – 191	3
☐	Beta strand	206 – 215	10
☐	Beta strand	218 – 220	3
☐	Helix	224 – 226	3
☐	Beta strand	228 – 237	10
☐	Beta strand	239 – 256	18
☐	Turn	257 – 260	4
☐	Beta strand	261 – 268	8
☐	Turn	269 – 271	3
☐	Beta strand	272 – 279	8

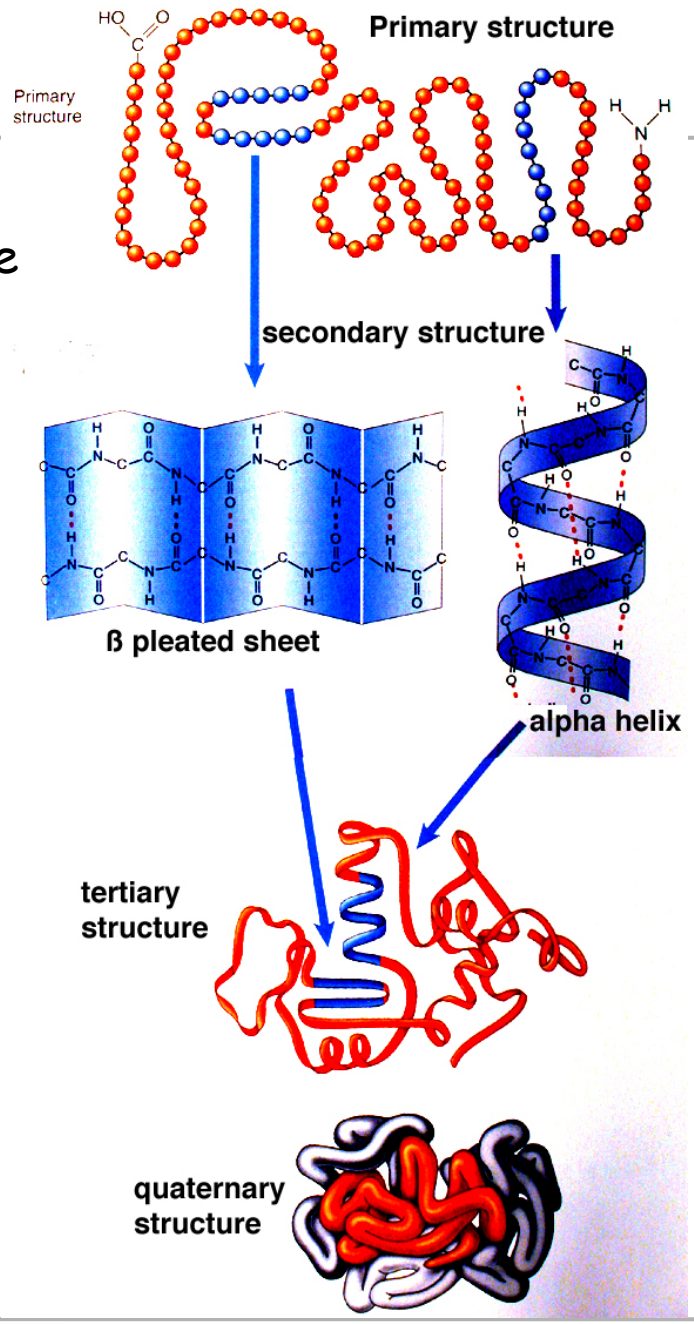
Protein structure

Primary structure: Amino acid sequence

Secondary structure:
"Backbone" hydrogen bonding
Alpha helix / Beta sheet / Turn

Tertiary structure: Fold, 3D coordinates

Quaternary structure: subunits



Evidence (Comments, Feature Table)

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Q43495 (108_SOLLC) Reviewed, UniProtKB/Swiss-Prot

Last modified March 2, 2010. Version 49. History...

General annotation (Comments)

Subcellular location	Secreted (Potential).
Tissue specificity	Stamen- and tapetum-specific.
Sequence similarities	Belongs to the A9/FIL1 family.

Sequence annotation (Features)

	Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Molecule processing						
☐	Signal peptide	1 – 30	30	Potential		
☐	Chain	31 – 102	72	Protein 108		PRO_0000000238
Amino acid modifications						
☐	Disulfide bond	41 ↔ 77		By similarity		
☐	Disulfide bond	51 ↔ 66		By similarity		
☐	Disulfide bond	67 ↔ 92		By similarity		
☐	Disulfide bond	79 ↔ 99		By similarity		

Evidence/Confidence types

3 types of *non-experimental qualifiers* in Sequence annotation and General comment:

- *Potential:* Predicted using sequence analysis
- *Probable:* Uncertain experimental evidence
- *By similarity:* Predicted using sequence similarity

UniProt entry, sequence(s)

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Sequences

	Sequence		Length	Mass (Da)	Tools
☐	**Isoform 1** [UniParc].	FASTA	418	46,737	Blast go

Last modified October 1, 1996. Version 3.
Checksum: 7016555F273B7F16

```
        10         20         30         40         50         60
MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS

        70         80         90        100        110        120
LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF

       130        140        150        160        170        180
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ

       190        200        210        220        230        240
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQVTTV

       250        260        270        280        290        300
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL

       310        320        330        340        350        360
ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA

       370        380        390        400        410
VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQK
```

« Hide

☐	**Isoform 2** [UniParc].	FASTA	359	40,263	Blast go

Checksum: D16A255538FB2945
Show »

☐	**Isoform 3** [UniParc].	FASTA	306	34,755	Blast go

Checksum: 15C708E6C25CE0C4
Show »

Cross-references, nucleotide sequences

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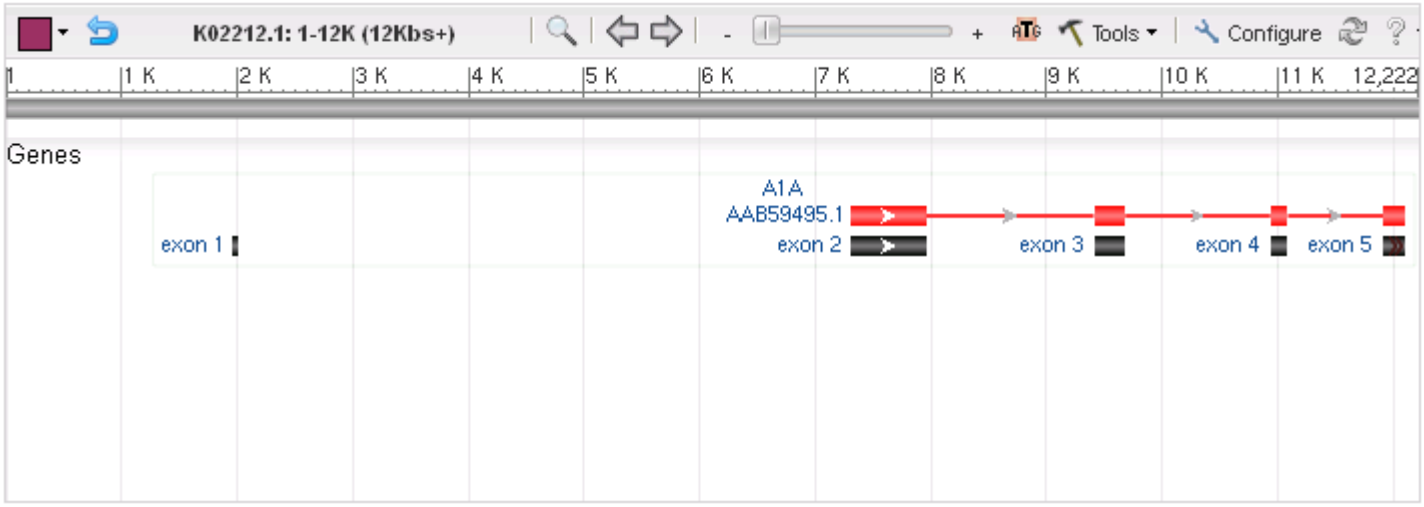
Sequence databases

- (•) EMBL
- ( ) GenBank
- ( ) DDBJ

K01396 mRNA. Translation: AAB59375.1.
K02212 Genomic DNA. Translation: AAB59495.1.
X01683 mRNA. Translation: CAA25838.1.
M11465 mRNA. Translation: AAA51546.1.
J02619 Genomic DNA. Translation: AAA51547.1.
DQ682455 mRNA. Translation: ABG73380.1.
AM048838 Genomic DNA. Translation: CAJ15161.1.
AF113676 mRNA. Translation: AAF29581.1.
AF130068 mRNA. Translation: AAG35496.1.
BX161449 mRNA. Translation: CAD61914.1.
BX247968 mRNA. Translation: CAD62306.1.
BX248002 mRNA. Translation: CAD62334.1. Different initiation.
BX248257 mRNA. Translation: CAD62585.1. Different initiation.
AK315637 mRNA. Translation: BAG38005.1.
BT019455 mRNA. Translation: AAV38262.1.



Cross-references, 3D structure

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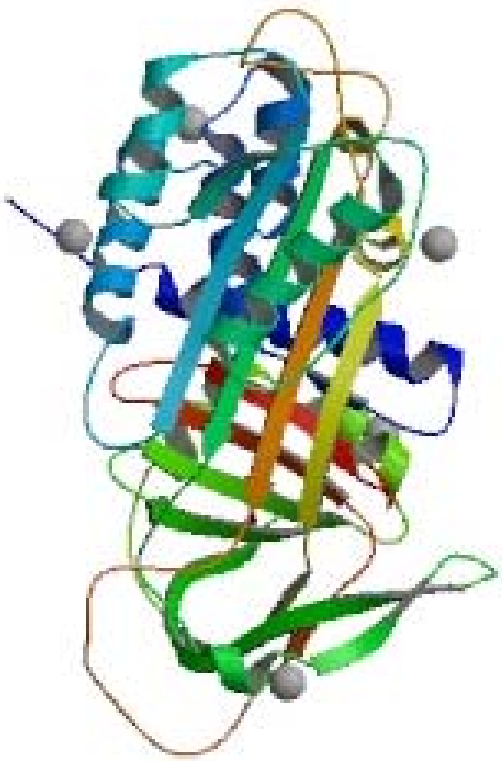
3D structure databases

- (•) PDBe
- ( ) RCSB PDB
- ( ) PDBj

Entry	Method	Resolution (Å)	Chain	Positions	PDBsum
1ATU	X-ray	2.70	A	45-418	[»]
1D5S	X-ray	3.00	A	44-377	[»]
			B	378-418	[»]
1EZX	X-ray	2.60	A	48-382	[»]
			B	383-418	[»]
1HP7	X-ray	2.10	A	25-418	[»]
1IZ2	X-ray	2.20	A	25-418	[»]
1KCT	X-ray	3.46	A	25-418	[»]
1OO8	X-ray	2.65	A	26-418	[»]
1OPH	X-ray	2.30	A	26-418	[»]
1PSI	X-ray	2.92	A	26-418	[»]
1QLP	X-ray	2.00	A	26-418	[»]
1QMB	X-ray	2.60	A	49-376	[»]
			B	377-418	[»]
2D26	X-ray	3.30	A	26-382	[»]
			B	383-418	[»]
2QUG	X-ray	2.00	A	25-418	[»]
3CWL	X-ray	2.44	A	25-418	[»]
3CWM	X-ray	2.51	A	25-418	[»]
3DRM	X-ray	2.20	A	26-418	[»]
3DRU	X-ray	3.20	A/B/C	26-418	[»]
3NDD	X-ray	1.50	A	46-372	[»]
			B	383-418	[»]
3NDF	X-ray	2.70	A	46-381	[»]
			B	383-418	[»]
3T1P	X-ray	3.90	A	48-418	[»]
7API	X-ray	3.00	A	36-382	[»]
			B	383-418	[»]
8API	X-ray	3.10	A	36-382	[»]
			B	383-418	[»]
9API	X-ray	3.00	A	36-382	[»]
			B	383-418	[»]



Cross-references

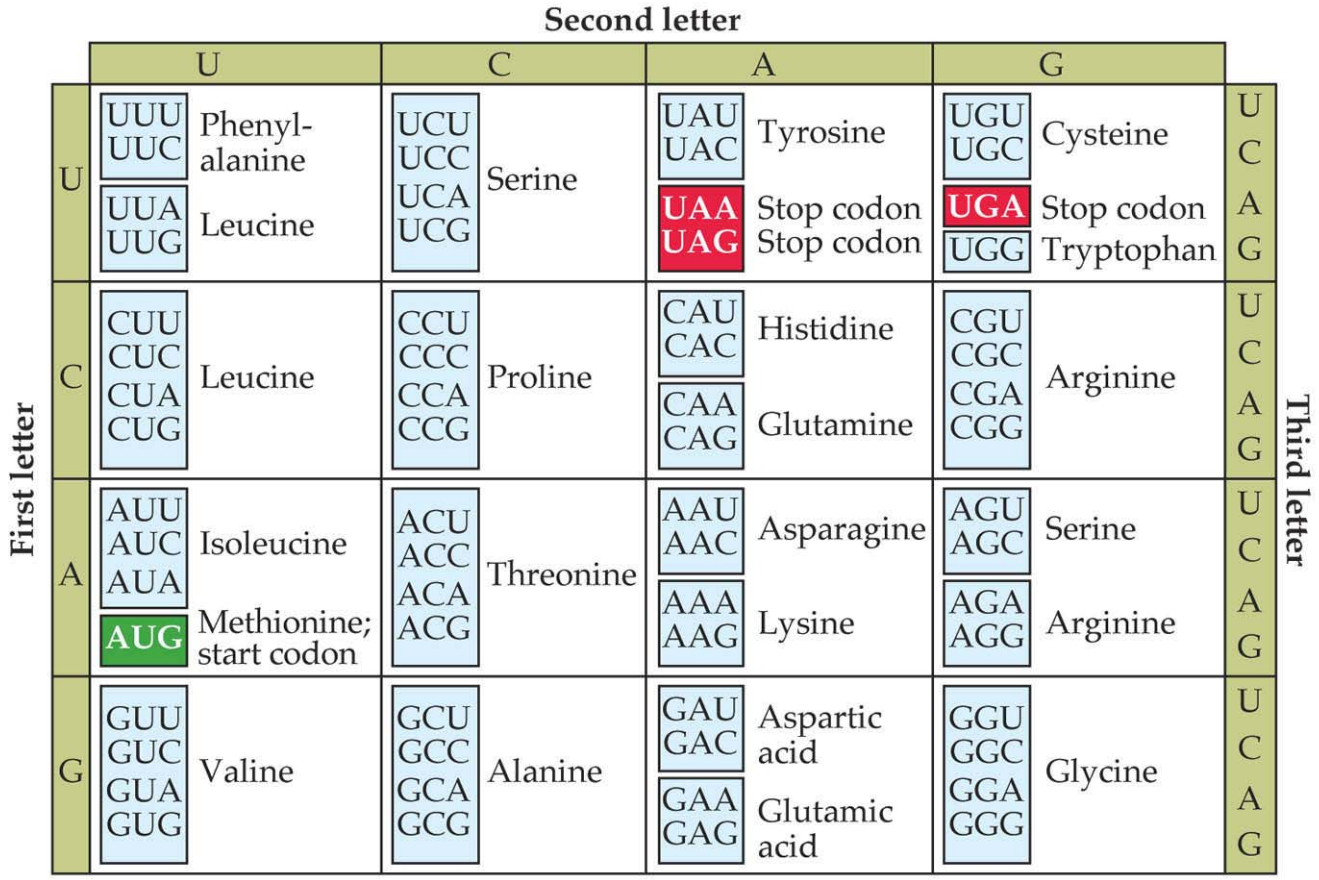
Other databases linked from UniProt

(there are ~100 in total):

- Nucleotide sequences
- 3D structure
- Protein-protein interactions
- Enzymatic activities and pathways
- Gene expression (microarrays and 2D-PAGE)
- Ontologies
- Families and domains
- Organism specific databases

Translation
and
Reading Frames

The genetic code



First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phenylalanine	UCU, UCC, UCA, UCG: Serine	UAU, UAC: Tyrosine	UGU, UGC: Cysteine	U C A G
	UUA, UUG: Leucine		UAA: Stop codon; UAG: Stop codon	UGA: Stop codon; UGG: Tryptophan	
C	CUU, CUC, CUA, CUG: Leucine	CCU, CCC, CCA, CCG: Proline	CAU, CAC: Histidine	CGU, CGC, CGA, CGG: Arginine	U C A G
			CAA, CAG: Glutamine		
A	AUU, AUC, AUA: Isoleucine	ACU, ACC, ACA, ACG: Threonine	AAU, AAC: Asparagine	AGU, AGC: Serine	U C A G
	AUG: Methionine; start codon		AAA, AAG: Lysine	AGA, AGG: Arginine	
G	GUU, GUC, GUA, GUG: Valine	GCU, GCC, GCA, GCG: Alanine	GAU, GAC: Aspartic acid	GGU, GGC, GGA, GGG: Glycine	U C A G
			GAA, GAG: Glutamic acid		

- Degenerate (*redundant*) but not ambiguous
- *Almost* universal (deviations found in mitochondria)

Reading Frames 1

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

A piece of an mRNA-strand:

```
5'                                                              3'
augcccaagcugaauagcguagaggggguuuucaucauuugaggacgauguauaa
```

can be divided into triplets (*codons*) in three ways:

```
1 aug ccc aag cug aau agc gua gag ggg uuu uca uca uuu gag gac gau gua uaa
   M   P   K   L   N   S   V   E   G   F   S   S   F   E   D   D   V   *
2  ugc cca agc uga aua gcg uag agg ggu uuu cau cau uug agg acg aug uau
    C   P   S   *   I   A   *   R   G   F   H   H   L   R   T   M   Y
3   gcc caa gcu gaa uag cgu aga ggg guu uuc auc auu uga gga cga ugu aua
     A   Q   A   E   *   R   R   G   V   F   I   I   *   G   R   C   I
```

Each possible set of triplets is called a *reading frame.*

Reading Frames 2

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

Since there are two strands in DNA, there are *six* possible reading frames in a piece of DNA (three in each direction):

```
 3    A  Q  A  E  *  R  R  G  V  F  I  I  *  G  R  C  I
 2   C  P  S  *  I  A  *  R  G  F  H  H  L  R  T  M  Y
 1  M  P  K  L  N  S  V  E  G  F  S  S  F  E  D  D  V  *
5' ATGCCCAAGCTGAATAGCGTAGAGGGGTTTTCATCATTTGAGGACGATGTATAA 3'
3' TACGGGTTCGACTTATCGCATCTCCCCAAAAGTAGTAAACTCCTGCTACATATT 5'
    H  G  L  Q  I  A  Y  L  P  K  *  *  K  L  V  I  Y  L  -1
      G  L  S  F  L  T  S  P  N  E  D  N  S  S  S  T  Y   -2
     A  W  A  S  Y  R  L  P  T  K  M  M  Q  P  R  H  I    -3
```

A reading frame from a start codon to the first stop codon is called an *open* reading frame (underlined above).